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# Study on the Effect of *Ficus exasperata* Aqueous Leaf Extract on the Rate of Lactic Acid Formation of Fermented Milk

[1]*Philip Idemudia Edogun*, [2]*Chinyere Imoisi and* [3]*Julius Uche Iyasele*

[1]*Department of Biological Sciences, Wellspring University, Edo State, Nigeria*

[2]*Department of Industrial Chemistry, Mewar International University, KM 21, Abuja-Keffi, Expressway, Masaka, Nasarawa State, Nigeria*

[3]*Department of Chemistry, University of Benin, Edo State, Nigeria*

## ABSTRACT

**Background and Objective:** The utilization of medicinal plant extracts, such as *Ficus exasperata*, in yoghurt production offers a novel avenue for creating functional dairy products with augmented nutritional and medicinal attributes. This study aimed to elucidate the fermentation kinetics and physicochemical characteristics of yoghurt fortified with *Ficus exasperata* leaf extract. **Materials and Methods:** Fermentation progress was monitored by measuring pH and titratable acidity (TA (%)) over a 12 hrs period at 43°C for yoghurt samples labeled Yc (control) and Y1 (fortified). Proximate composition analysis encompassing moisture content, total solid content, specific gravity, ash content and protein content was conducted to evaluate the impact of fortification on nutritional parameters. Furthermore, the kinetics of lactic acid formation during fermentation were assessed using a differential method to discern reaction order and rate constant. **Results:** The prolonged fermentation time for fortified yoghurt samples (Y1, Y2 and Y3) compared to the control (Yc). Lactic acid formation followed a fractional order kinetics, suggesting intricate biochemical processes during fermentation. Physicochemical analysis revealed variations in pH, titratable acidity, moisture content, total solids, ash content, protein content and specific gravity among yoghurt variants. Additionally, the pH stability of all yoghurt samples was monitored for eight days. **Conclusion:** These findings underscore the potential of *Ficus exasperata* fortified yoghurt in offering enhanced nutritional value and bioactive properties, aligning with consumer preferences for healthier dietary options.

## KEYWORDS

Fermentation, yoghurt, *Ficus exasperate*, pH, proximate composition and titratable acidity



## INTRODUCTION

The growing global consumption of fermented milk products, particularly yoghurt, can be attributed to various factors, including its perceived convenience and nutritional benefits[1]. Yoghurt is considered a convenient source of protein and is often preferred by individuals with lactose intolerance due to its pre-digested nature. Moreover, it is recognized as a dietary staple that provides essential components for body maintenance and defense[2].

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The concept of "right food" is crucial in understanding consumer preferences, encompassing characteristics such as attractive appearance, pleasant flavor, affordability and inclusion of natural ingredients[3]. As dietary habits evolve, there is a growing demand for foods that offer nutritional value, are low in fat and contain bioactive compounds known to reduce disease risk[4].

Yoghurt, a fermented dairy product, has been consumed worldwide for its nutritional value and health benefits. Its production involves the fermentation of milk by lactic acid bacteria, primarily *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, leading to the formation of lactic acid, which imparts the characteristic tangy flavor and acidic taste to yoghurt[5,6].

In recent years, there has been growing interest in fortifying yoghurt with bioactive compounds derived from plant sources to enhance its functional properties and health-promoting effects. *Ficus exasperata*, a plant commonly found in tropical regions, has gained attention for its potential therapeutic properties, including antioxidant, antimicrobial and antihypertensive activities[7]. Yoghurt production involves fermenting milk, typically from cows but occasionally from other animals, using microorganisms with economic significance in food processing, such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*[8]. The fermentation process enhances the nutritional content of milk and contributes to the tangy flavor characteristic of yoghurt.

Recent advancements in yoghurt production involve enriching the product with natural plant species to enhance its nutritional quality. These plant species, including grapes, strawberries and apples, can accelerate or decelerate the fermentation process and provide additional vitamins and minerals[9]. Some plants, like *Ficus exasperata*, possess medicinal properties, such as hypoglycemic and antihypertensive effects, which can be incorporated into yoghurt to promote overall health.

*Ficus exasperata*, commonly known as sandpaper leaf, is a medicinal plant native to Afro-tropical regions, known for its therapeutic benefits in traditional medicine[10,11]. The plant contains compounds like tannins, flavonoids and saponins, which contribute to its medicinal properties[12,13]. The addition of *Ficus exasperata* leaf extract to fermented milk serves as a carrier for its active components, offering consumers a convenient way to benefit from its medicinal properties. This approach aligns with consumer preferences for functional foods that promote health and well-being[14].

In summary, the incorporation of medicinal plant extracts like *Ficus exasperata* into yoghurt production represents an innovative approach to creating functional dairy products with enhanced nutritional and medicinal benefits. This research aims to investigate the fermentation rate of milk with *Ficus exasperata* leaf extract and compare the properties of yoghurt fortified with these extracts to traditional yoghurt.

## MATERIALS AND METHODS

**Collection of plant material:** Fresh leaves of *Ficus exasperata* were harvested from the Ugbowo Campus of the University of Benin, located in Benin City, Edo State. Subsequently, the leaves were sent to the Department of Forestry and Wildlife at the University of Benin for identification purposes. This study was carried out from February, 2010 to July, 2011.

**Preparation of yoghurt culture starter:** A quantity of 5 g of dried powder culture starter containing the three microorganisms-*Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus*, along with sucrose and ascorbic acid, was dissolved in 1000 mL of water at room temperature. This solution was prepared in a 1000 mL volumetric flask and then stored in the laboratory for 3 days to allow the microorganisms to become active[15].

**Preparation of plant material and extract:** The collected leaves *of Ficus exasperata* were air-dried naturally at room temperature over a period of ten days. Subsequently, they were pulverized into a powdered form using a mortar and pestle, followed by filtration through a mesh. For the extraction process, a cold extraction method was employed. Portions of 1, 2 and 3 g of the powdered *Ficus exasperata* leaf were separately macerated in 100 milliliters of distilled water within a 250 mL beaker. The mixture was then filtered under constant stirring using sintered glass. After filtration, the extract obtained was stored in a refrigerator until further use[16].

**Preparation of yoghurt starter culture:** Initially, 20 g of skimmed milk was combined with 200 mL of water. This process was conducted to produce four distinct samples, denoted as Yc, Y1, Y2 and Y3. These labels correspond to control yoghurt (without the plant extract), fortified yoghurt containing 10 mL (1 g in 100 mL) of plant extract, fortified yoghurt containing 20 mL (2 g in 200 mL) of plant extract and fortified yoghurt containing 30 mL (3 g in 100 mL) of plant extract, respectively[17].

For the control yoghurt, the 20 g of milk-water mixture (200 mL) was thoroughly stirred and then pasteurized at 78°C for 18 min. Subsequently, it was cooled to 43°C before inoculation with a prepared cultured starter solution (10 mL added to the pasteurized milk). The mixture was then incubated for over 8 hrs to facilitate coagulation. Finally, the yoghurt was cooled in a water bath to room temperature and refrigerated at a temperature below 20°C. The resulting product was transferred to a plastic container for further analysis. This procedure was replicated for the production of Y1, Y2 and Y3. In each case, the plant's leaf extract was added to the milk-water mixture before pasteurization and inoculation. For Y1 production, 10 mL of the plant extract was added, while 20 mL and 30 mL were added for Y2 and Y3 production, respectively. After pasteurization and inoculation, the mixtures were incubated until the desired yoghurt pH of 4.2 was achieved. These processes were conducted overnight in the laboratory, during which the rate of lactic acid formation from lactose by lactic acid bacteria was monitored for approximately 13 hrs. Finally, the yoghurt samples were cooled and transferred to plastic bottles for further analysis.

**Analysis of fermented milk (yoghurt):** As previously mentioned, the yoghurts created were categorized as Yc (control yoghurt), Y1 (10 mL of 1 g per 100 mL), Y2 (20 mL of 2 g per 100 mL) and Y3 (30 mL of 3 g per 100 mL). Subsequently, these yoghurt samples underwent analysis to assess their physiochemical properties, including pH, titratable acidity, protein content, specific gravity, moisture content and total solid content[17].

**pH determination:** Throughout the yoghurt production process, the pH of the samples was monitored and documented. This involved utilizing a calibrated pHeb pH meter, which was inserted into each yoghurt sample hourly to measure and record the pH levels.

**Titratable acidity determination:** This involved setting up a titration apparatus consisting of a retort stand, clamp, burette (50 mL) and the use of a syringe (5 mL). A solution of phenolphthalein indicator and 0.1 M sodium hydroxide was prepared in the laboratory by dissolving 4 g of sodium hydroxide in 1000 mL of distilled water in a volumetric flask. The burette was then filled with the sodium hydroxide solution up to the 50 mL mark using a funnel, clamp and retort stand. The 0.1 M sodium hydroxide solution was titrated against the lactic acid produced from the lactose in the yoghurt samples. The addition of phenolphthalein indicator facilitated the observation of a color change to pink, indicating neutralization. This process was repeated for each yoghurt sample (Yc, Y1, Y2 and Y3) and the volume of sodium hydroxide required to neutralize the lactic acid was recorded to measure the titratable acidity[18].

**Determination of specific gravity:** The specific gravity of the samples was determined using a specific gravity bottle (25 mL). Firstly, the weight of a given volume of each sample was measured on a balance. Then, the weight of the same volume of water was measured and recorded. The specific gravity was calculated by comparing the weight of the sample to the weight of an equal volume of water[18].

**Determination of moisture content:** Moisture was eliminated from the yoghurt samples by employing an oven set at a temperature of 110°C for 1¾ hrs. Initially, the crucibles were weighed empty, then weighed again after adding the yoghurt sample before placing them into the oven as described. After 1¾ hrs, the samples were transferred into a desiccator containing silica gel to absorb any residual moisture. Once removed from the desiccator, the samples along with the crucibles were reweighed and then returned to the oven. This process was repeated three times until a consistent weight was achieved and recorded for samples Yc, Y1, Y2 and Y3. The difference in weight was then used to determine the moisture content[19].

**Total solid content:** This involves using an oven set at the same temperature and duration as specified for moisture content, with the difference being that it focuses on the residue remaining after moisture removal.

**Determination of ash content:** The crucibles were first subjected to an oven at 105°C for 40 min. Subsequently, they were cooled to room temperature within a desiccator and then weighed. Next, 5 g of each yoghurt product (Yc, Y1, Y2 and Y3) was placed into individual crucibles and transferred into a muffle furnace at a temperature of 520°C until completely devoid of carbon residue. This was confirmed when no black particles remained in the food sample. The crucibles were then reweighed and the data recorded, from which the amount of ash was calculated for each sample of yoghurt labeled accordingly[19].

**Determination of crude protein:** The determination of crude protein involved several steps, beginning with the initial determination of the percentage of nitrogen multiplied by a conversion factor for dairy products, such as milk, by 6.38. Three crucial steps were followed during this experiment: Digestion of yoghurt samples, distillation and titration. Dried samples of yoghurt, from which moisture had been removed, were utilized, as the experiment necessitated dried samples. These samples were ground to a fine powdered form and carefully mixed to homogenize them before being accurately weighed to 1.000±0.002 g, approximately 1 g.

During the digestion process, 0.30 g of yoghurt samples were precisely weighed and placed in micro Kjeldahl test tubes labeled for each sample (Yc, Y1, Y2 and Y3). Subsequently, one tablet of Kjeltab auto (containing 1.5 g $K_2SO_4$ and 7.5 mg Se) was added. Potassium sulphate ($K_2SO_4$) was added to raise the boiling point, facilitating decomposition, while selenium (Se) acted as the catalyst. Next, 12 mL of concentrated sulphuric acid ($H_2SO_4$) was carefully added and the tubes were heated to 420°C for 30 min under a fume hood. After 30 min, the tubes were removed, cooled and diluted with 75 mL of distilled water before distillation. During distillation, a receiver solution (containing 25 mL of 0.1 N $H_2SO_4$ and 25 mL distilled water) was prepared in a 250 mL volumetric flask to collect the distillate. The digested products were transferred into distillation tubes and subjected to distillation. About 75 mL of 35% sodium hydroxide (NaOH) solution was added to the distillation tube and distillation commenced immediately. Approximately 150-200 mL of distillate was collected in the receiver before titration. The distillation process allowed the ammonium solution to distil into a conical flask. Finally, the distillate was titrated with 0.1N NaOH standard solution using ten drops of Bromo cresol green indicator until a clear orange coloration solution, indicating the endpoint, was observed. These procedures were conducted for the four samples (Yc, Y1, Y2 and Y3), as well as a blank. The experiments were repeated and the average value of nitrogen, as well as the protein content, was calculated using the formula[19]:

$$\text{Protein content (\%)} = \frac{(A - B) \times N \times 14.007 \times \text{Volume (mL)} \times 100 \times 6.38}{\text{Weight of the sample (mg)}}$$

**Statistical analysis:** Statistical analysis was carried out with the statistical package BMDP, using the BMDP 2R program (stepwise multiple regression). Results were expressed as mean of triplicate analysis.

## RESULTS AND DISCUSSION

The results presented in Table 1 depicted the pH, volume (mL) and percentage titratable acidity (TA (%)) of yoghurt samples labeled as Yes (control) and Y1 at various time intervals during fermentation.

- **Time (hr):** Time elapsed during fermentation process
- **pH:** pH values of yoghurt samples at each time interval
- **Vol (mL):** Volume of yoghurt samples measured in milliliters
- **TA (%):** Percentage of titratable acidity in yoghurt samples

These measurements provide insights into the fermentation kinetics and physicochemical changes occurring during the fermentation process, facilitating the assessment of the impact of *Ficus exasperata* leaf extract fortification on yoghurt characteristics.

The data presented in Table 2 illustrated the pH, volume (mL) and percentage titratable acidity (TA (%)) of yoghurt samples labeled as Y2 and Y3 throughout the 12 hrs fermentation process at 43°C.

- **Time (hr):** Duration of fermentation process
- **pH:** pH values of yoghurt samples at each time point
- **Vol (mL):** Volume of yoghurt samples measured in milliliters
- **(TA (%)):** Percentage of titratable acidity in yoghurt samples

This data enables the assessment of the fermentation kinetics and physicochemical changes occurring in yoghurt samples fortified with different concentrations of *Ficus exasperata* leaf extract.

The data provided in Table 3 presented the physicochemical properties of yoghurt samples denoted as Yc, Y1, Y2 and Y3, including pH, titratable acidity (%), moisture content (%), total solid content (%), specific gravity, ash content (%) and protein content (%).

- **pH:** Represents the acidity or alkalinity of the yoghurt samples
- **Titratable acidity (%):** Percentage of titratable acidity, indicating the acidity level of yoghurt
- **Moisture content (%):** Percentage of water content present in the yoghurt samples
- **Total solid content (%):** Percentage of non-water components present in the yoghurt samples
- **Specific gravity:** Density of the yoghurt samples relative to water
- **Ash content (%):** Percentage of inorganic residue remaining after complete combustion of yoghurt samples
- **Protein content (%):** Percentage of protein present in the yoghurt samples

These parameters provide valuable insights into the nutritional composition and quality characteristics of yoghurt fortified with *Ficus exasperata* leaf extract.

Table 4 illustrated the kinetics of lactic acid formation during the fermentation of yoghurt, showing the percentage of titratable acidity (TA (%)), rate of lactic acid formation (V = C/t) and their corresponding logarithmic values.

Table 5 displayed the kinetics of lactic acid formation during the fermentation of yoghurt, showcasing the percentage of titratable acidity (TA (%)), rate of lactic acid formation (V = C/t) and their corresponding logarithmic values.

Table 6 displayed the kinetics of lactic acid formation during the fermentation of yoghurt, showcasing the percentage of titratable acidity (TA (%)), rate of lactic acid formation (V = C/t) and their corresponding logarithmic values.

Table 1: Values for pH and titratable acidity (%) with time for every 1 hr at 43°C for Yc and Y1

| | Samples | | | | | |
|---|---|---|---|---|---|---|
| | Yes | | | Y1 | | |
| Time (hr) | pH | Vol (mL) | TA (%) | pH | Vol (mL) | TA (%) |
| 0 | 6.60±0.10 | 1.15±0.07 | 0.21±0.01 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 1 | 6.60±0.10 | 1.15±0.07 | 0.21±0.01 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 2 | 6.60±0.10 | 1.15±0.07 | 0.21±0.01 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 3 | 6.40±0.10 | 1.25±0.07 | 0.23±0.01 | 6.60±0.10 | 0.10±0.07 | 0.10±0.01 |
| 4 | 6.40±0.10 | 1.30±0.07 | 0.23±0.11 | 6.40±0.10 | 1.30±0.14 | 0.23±0.03 |
| 5 | 6.30±0.10 | 1.50±0.14 | 0.27±0.03 | 6.40±0.10 | 1.30±0.14 | 0.23±0.03 |
| 6 | 5.80±0.10 | 2.00±0.14 | 0.36±0.03 | 5.90±0.10 | 2.10±0.07 | 0.38±0.01 |
| 7 | 5.00±0.10 | 4.30±0.21 | 0.77±0.04 | 5.00±0.10 | 2.80±0.14 | 0.50±0.03 |
| 8 | 4.60±0.10 | 5.50±0.14 | 0.99±0.03 | 4.90±0.10 | 3.10±0.28 | 0.56±0.05 |
| 9 | 4.90±0.10 | 5.80±0.07 | 1.04±0.01 | 4.60±0.10 | 4.80±0.14 | 0.86±0.03 |
| 10 | 4.20±0.10 | 6.90±0.35 | 1.24±0.06 | 4.40±0.10 | 5.20±0.28 | 0.94±0.05 |
| 11 | 4.10±0.10 | 8.60±0.21 | 1.55±0.04 | 4.30±0.10 | 6.90±0.07 | 1.24±0.01 |
| 12 | | | | 4.10±0.10 | 7.60±0.42 | 1.37±0.08 |
| 13 | | | | 4.10±0.10 | 7.60±0.42 | 1.37±0.08 |

±: Margin of error or uncertainty in the reported values

Table 2: Values for pH and titratable acidity (%) with time for every 1 hr at 43°C for Y2 and Y3

| | Y2 | | | Y3 | | |
|---|---|---|---|---|---|---|
| Time (hr) | pH | Vol (mL) | TA (%) | pH | Vol (mL) | TA (%) |
| 0 | 6.60±0.10 | 1.10±0.07 | 0.19±0.07 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 1 | 6.60±0.10 | 1.10±0.07 | 0.19±0.07 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 2 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 3 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 | 6.60±0.10 | 1.10±0.07 | 0.19±0.01 |
| 4 | 6.40±0.20 | 0.23±0.07 | 6.40±0.10 | 1.40±0.14 | 0.25±0.23 | 0.25±0.23 |
| 5 | 6.40±0.10 | 1.30±0.07 | 0.23±0.01 | 6.40±0.10 | 1.40±0.21 | 0.25±0.04 |
| 6 | 6.0±0.10 | 2.10±0.11 | 0.36±0.02 | 6.20±0.10 | 1.60±0.11 | 0.29±0.02 |
| 7 | 5.40±0.10 | 3.20±0.07 | 0.58±0.01 | 5.80±0.10 | 3.20±0.07 | 0.58±0.01 |
| 8 | 5.00±0.10 | 4.0±0.14 | 0.74±0.03 | 5.50±0.10 | 3.20±0.07 | 0.58±0.01 |
| 9 | 4.80±0.10 | 4.90±0.11 | 0.88±0.02 | 5.20±0.10 | 4.10±0.14 | 0.74±0.03 |
| 10 | 4.70±0.10 | 5.10±0.14 | 0.92±0.03 | 4.90±0.10 | 5.10±0.14 | 0.92±0.03 |
| 11 | 4.60±0.10. | 6.00±0.14 | 1.08±0.03 | 4.70±0.10 | 5.30±0.07 | 0.95±0.01 |
| 12 | 4.40±0.10 | 6.80±0.21 | 1.22±0.04 | 4.60±0.10 | 6.10±0.21 | 1.09±0.04 |
| 13 | 4.20±0.10 | 7.40±0.11 | 1.33±0.02 | 4.30±0.10 | 7.20±0.14 | 1.29±0.03 |

±: Margin of error or uncertainty in the reported values

Table 3: Physicochemical properties of yoghurt samples (Yc, Y1, Y2 and Y3) fortified with *Ficus exasperata* leaf extract

| C | Yc | $Y_1$ | $Y_2$ | $Y_3$ |
|---|---|---|---|---|
| pH | 4.10±0.10 | 4.10±0.10 | 4.20±0.10 | 4.40±0.10 |
| Titratable acidity (%) | 1.55±0.04 | 1.37±0.02 | 1.33±0.02 | 1.29±0.03 |
| Moisture content (%) | 86.07±1.46 | 90.33±3.66 | 91.39±3.24 | 92.36±3.12 |
| Total solid content (%) | 13.93±1.46 | 9.67±3.66 | 8.62±3.24 | 7.64±3.12 |
| Specific gravity | $1.02\pm(1.00)\times10^{-4}$ | $1.01\pm(1.70)\times10^{-3}$ | $1.01\pm(3.00)\times10^{-4}$ | $1.01\pm(1.30)\times10^{-3}$ |
| Ash content (%) | 0.75±0.02 | 0.76±0.01 | 0.79±0.02 | 0.82±0.03 |
| Protein content (%) | 9.00±0.96 | 3.65±1.47 | 3.64±0.74 | 2.46±1.79 |

±: Margin of error or uncertainty in the reported values

Table 4: Kinetics of lactic acid formation during yoghurt fermentation for yoghurt sample Yc

| Time (hr) | C = TA (%) | V = C/t | Log C | Log V |
|---|---|---|---|---|
| 2 | 0.210 | 0.125 | -0.678 | -0.979 |
| 4 | 0.230 | 0.058 | -0.638 | -1.240 |
| 6 | 0.360 | 0.060 | -0.444 | -1.222 |
| 8 | 0.990 | 0.124 | 0.004 | -0.907 |
| 10 | 1.240 | 0.124 | 0.093 | -0.889 |
| 12 | 1.550 | 0.124 | 0.190 | -0.889 |

Table 5: Kinetics of lactic acid formation during yoghurt fermentation yoghurt sample Y1

| Time (hr) | C = TA (%) | V = C/t | Log C | Log V |
|---|---|---|---|---|
| 2 | 0.190 | 0.095 | -0.721 | -1.022 |
| 4 | 0.230 | 0.058 | -0.638 | -1.237 |
| 6 | 0.380 | 0.063 | -0.420 | -1.201 |
| 8 | 0.560 | 0.070 | -0.252 | -1.151 |
| 10 | 0.940 | 0.094 | -0.027 | -1.027 |
| 12 | 1.370 | 0.114 | 0.136 | -0.866 |
| 14 | 1.370 | 0.098 | 0.136 | -1.009 |

Table 6: Kinetics of lactic acid formation during yoghurt fermentation yoghurt sample $Y_2$

| Time (hr) | C = TA (%) | V = C/t | Log C | Log V |
|---|---|---|---|---|
| 2 | 0.190 | 0.095 | -0.721 | -1.022 |
| 4 | 0.230 | 0.058 | -0.638 | -1.237 |
| 6 | 0.360 | 0.060 | -0.444 | -1.222 |
| 8 | 0.740 | 0.093 | -0.131 | -1.032 |
| 10 | 0.920 | 0.092 | -0.036 | -1.036 |
| 12 | 1.220 | 0.102 | 0.086 | -0.990 |
| 14 | 1.330 | 0.095 | 0.124 | -0.907 |

Table 7: Kinetics of lactic acid formation during yoghurt fermentation yoghurt sample $Y_3$

| Time (hr) | C = TA (%) | V = C/t | Log C | Log V |
|---|---|---|---|---|
| 2 | 0.190 | 0.095 | -0.721 | -1.022 |
| 4 | 0.250 | 0.063 | -0.602 | -1.201 |
| 6 | 0.290 | 0.048 | -0.538 | -1.319 |
| 8 | 0.590 | 0.074 | -0.229 | -1.131 |
| 10 | 0.920 | 0.092 | -0.0362 | -1.036 |
| 12 | 1.090 | 0.091 | 0.0374 | -1.041 |
| 14 | 1.290 | 0.092 | 0.111 | -1.036 |

Table 7 displayed the kinetics of lactic acid formation during the fermentation of yoghurt, showcasing the percentage of titratable acidity (TA (%)), rate of lactic acid formation (V = C/t) and their corresponding logarithmic values.

In this study, the fermentation process and physiochemical properties of yoghurt fortified with *Ficus exasperata* leaf extract were investigated. The process involved monitoring pH, titratable acidity (TA (%)) and other proximate composition parameters over time to understand the effects of fortification on yoghurt quality and stability[20,21].

The pH and TA (%) values were monitored hourly during fermentation for yoghurt samples labeled Yc, Y1, Y2 and Y3, maintained at 43°C. Initially, all samples showed similar pH values, indicating a neutral environment, while TA (%) remained relatively low[22]. However, as fermentation progressed, there were notable changes in both pH and TA (%) In general, the pH decreased gradually over time, indicating increasing acidity due to lactic acid production. This decline in pH was more pronounced in fortified yoghurt samples (Y1, Y2 and Y3) compared to the control (Yc), suggesting that the addition of *Ficus exasperata* leaf extract accelerated the fermentation process[23,24]. Furthermore, the TA (%) values increased steadily throughout the fermentation period for all samples, reflecting the accumulation of lactic acid as a by-product of bacterial metabolism. Notably, the fortified yoghurt samples exhibited higher TA (%) compared to the control, indicating enhanced acidity resulting from the presence of the plant extract[25].

The proximate composition analysis revealed significant differences among the yoghurt samples. Moisture content was higher in fortified yoghurt samples (Y1, Y2 and Y3) compared to the control (Yc), likely due to the addition of the aqueous leaf extract. This increase in moisture content could influence the texture and shelf life of the fortified yoghurt products, necessitating the use of stabilizers or thickeners to mitigate microbial spoilage[26,27]. Moreover, the total solid content showed a decreasing trend with fortification, indicating dilution of the yoghurt matrix by the aqueous extract. This observation underscores the importance of maintaining proper balance in the formulation to ensure the desired product consistency and nutritional profile. The specific gravity and ash content increased with fortification, suggesting the incorporation of mineral components from the plant extract into the yoghurt matrix. This enrichment may confer additional health benefits and contribute to the overall nutritional value of the fortified yoghurt products. Additionally, the protein content decreased with fortification, indicating a dilution effect caused by the addition of the aqueous extract[28]. However, the fortified yoghurt samples still contained substantial levels of protein, albeit lower than the control, highlighting the importance of fortification in maintaining nutritional adequacy[29].

The kinetics of lactic acid formation during fermentation were assessed using a differential method, revealing fractional orders of reaction for all yoghurt samples. This complexity in the reaction kinetics suggests the involvement of multiple biochemical processes, further emphasizing the need for comprehensive understanding and optimization of the fermentation process[30]. Overall, the findings of this study demonstrate the feasibility of fortifying yoghurt with *Ficus exasperata* leaf extract to enhance its nutritional value and health benefits. However, careful formulation and process optimization are essential to ensure product quality, stability and consumer acceptance. Further research is warranted to explore the potential synergistic effects of fortification on yoghurt functionality and bioactivity[31].

The three microorganisms, namely *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus*, became active within three days after the preparation of the culture starter. This activation was facilitated by the sucrose present in the powder starter[31]. Physiochemical analysis conducted on the yoghurt samples produced at 43°C revealed notable findings. The yoghurt sample without fortification (Yc) exhibited higher acidity, with a mean pH value of 4.10 and a titratable acidity (TA (%)) of 1.55%. In contrast, samples Y1, Y2 and Y3 showed pH values and TA (%) of 4.30 and 0.95%, 4.60 and 1.08% and 4.70 and 0.95%, respectively, after 11 hrs. Subsequently, after 13 hrs, the pH and TA (%) for samples Y1, Y2 and Y3 were recorded as 4.10 and 1.37%, 4.20 and 1.33% and 4.30 and 1.29%, respectively. The prolonged fermentation period for samples Y1, Y2 and Y3 can be attributed to the effect of *Ficus exasperata* leaf extract, which minimally influenced the culture starter (microorganisms). The observed pH ranges were comparable to those reported by previous studies[32].

Moisture content analysis indicated that fortified yoghurt samples (Y1, Y2 and Y3) exhibited higher moisture content compared to plain yoghurt (Yc). This increase in moisture content in fortified yoghurt samples was due to the liquid extract of the plant added. The high moisture content contributed to controlling viscosity and texture while promoting the softness of the yoghurt samples. However, it also posed a risk of quick spoilage, which could be mitigated by adding stabilizers or thickeners to enhance shelf life[32]. Total solid content analysis revealed a decrease in mean values for plain yoghurt (Yc) compared to fortified yoghurt (Y1, Y2 and Y3). The values obtained were within the range reported by previous studies, suggesting satisfactory results. The percentage of specific gravity and ash content increased from plain yoghurt to fortified yoghurt, indicating the addition of mineral components to the milk product[33,34].

Protein content analysis showed varying percentages for samples Yc, Y1, Y2 and Y3, with fortified yoghurt samples exhibiting lower protein content compared to plain yoghurt. This lower protein content correlated with the lower total solids observed. The change in pH and TA (%) with time, illustrated the fermentation process's start and end. The fermentation process for yoghurt fortified with *Ficus exasperata* leaf extract took a longer time compared to plain yoghurt. The determination of the order of reaction for the fermentation process was conducted, with fractional values obtained for plain yoghurt and fortified yoghurt, indicating a complex reaction. The pH and TA (%) were recorded over eight days, with the storage temperature effectively preventing or slowing down yeast growth. Additionally, the decrease in total solid content corresponded to an increase in moisture content for the yoghurt samples[35,36].

## CONCLUSION

This study demonstrates the feasibility of producing high-quality yoghurt fortified with *Ficus exasperata* leaf extract using low-fat milk powder. It is plausible that the combination of low-fat milk powder and the leaf extract employed in yoghurt production may stimulate muscarinic receptors in the heart, leading to an initial decrease in cardiac output and subsequently, low blood pressure. Another possibility is that the presence of the plant extract may trigger the release of histamine into the circulatory system, resulting in a decrease in blood pressure via reduced peripheral resistance. High-quality yoghurt can be crafted by enriching it with a water extract derived from *Ficus exasperata* leaf, which has been assessed for its antihypertensive properties, provided that a meticulous fermentation process is adhered to. However, to ensure the stability of such fortified yoghurt, it is imperative to include stabilizers or thickeners, which not only enhance the texture but also reduce moisture content, thereby mitigating microbial spoilage. Furthermore, since certain nutrients like proteins and vitamins may be lost during processing, it is essential to fortify the yoghurt products with additional proteins, vitamins and minerals to maintain their overall nutritional value and enhance their general acceptability.

## SIGNIFICANCE STATEMENT

This study investigates the impact of incorporating *Ficus exasperata* leaf extract into yoghurt production, aiming to create functional dairy products with enriched nutritional and medicinal benefits. The objective is to analyze fermentation kinetics and physicochemical properties of yoghurt fortified with the extract. Fermentation time was extended for fortified yoghurt samples (Y1, Y2 and Y3) compared to control (Yc). Lactic acid formation followed a fractional order, suggesting complex reactions during fermentation. Physicochemical analysis revealed differences in pH, titratable acidity, moisture content, total solids, ash content, protein content and specific gravity among yoghurt variants. The study highlights the potential for developing functional dairy products with improved nutritional and bioactive properties through *Ficus exasperata* leaf extract fortification. These findings contribute to meeting consumer demand for healthier food choices.

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